Docket No. RDID 0073 US *PATENT*

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of: Mueller, Rainer, *et al.*

Application No.: To Be Assigned Group No.: To Be Assigned

Filed: July 23, 2001 Examiner: To Be Assigned

For: EXPRESSION OF ALKALINE PHOSPHATASE IN YEAST

Assistant Commissioner for Patents
Washington, D.C. 20231

PRELIMINARY AMENDMENT

Sir:

Please enter the following amendments prior to examination of the above-referenced application:

IN THE CLAIMS:

Please enter the following amendments to claims 1 through 15 as filed in the originally filed application. Both a clean and a marked-up copy of the claims as amended are attached.

Respectfully submitted,

Date: July 23, 2001

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Claims

1. A process for the production of a eukaryotic alkaline phosphatase in yeast cells comprising the steps: a) cloning [a] an alkaline phosphatase gene sequence into different vectors b) transformation of the yeast, c) expression of the alkaline phosphatase and d) purification of the alkaline phosphatase, wherein
   - a first vector is utilized that has a resistance gene for a first selection marker,
   - transformants are used which have integrated the resistance gene and the gene sequence into the genome, said transformants being [are] selected by growth on nutrient medium containing a low concentration of a first selection marker,
   - the gene copy number is increased by multiple transformation in which multiple transformants are selected by growth on nutrient medium at an increased selection pressure,
   - a second vector is [added] used which has a resistance gene for a second selection marker,
   - the gene copy number is increased by multiple transformation with the second vector in which multiple transformants are selected by growth on nutrient medium at an increased selection pressure and
   - those [clones] tranformants [are selected] which have integrated several copies of the gene sequence and the selection marker resistance genes in a stable manner are selected for producing the eukaryotic alkaline phosphatase.

2. The process according to claim 1 [the invention], wherein the gene sequence for the alkaline phosphatase corresponds to SEQ ID NO:1.

3. The process as claimed in [one of the claims 1 or 2] claim 1, wherein the alkaline phosphatase gene sequence corresponds to SEQ ID NO:5.

4. The process as claimed in [one of the claims 1 to 3] claim 1, wherein methylotrophic yeast cells are used.

5. The process as claimed in [one of the claims 1 to 4] claim 1, wherein Pichia pastoris or Hansenula polymorpha is used as the yeast strain.

6. The DNA sequence according to SEQ ID NO:5.

7. A transformation vector containing SEQ ID NO:5.

8. The vector as claimed in claim 7, which essentially corresponds to pHAP10-3.

9. A vector [containing] comprising the [entire] expression cassette from pHAP10-3.

10. The vector as claimed in claim 9, which essentially corresponds to pHAP10-3/9K.

11. A host strain transformed with a vector as claimed in claim 9 or 10.

12. A host strain transformed with the vector pHAP10-3/9K and/or a vector as claimed in claims 7 or 8.

13. The host strain as claimed in claim 12, wherein Pichia pastoris or Hansenula polymorpha is used as the host strain.

14. A Pichia pastori X-33 yeast strain transformed with a vector as claimed in claims 8 to 10.

15. A process for producing a eukaryotic highly active alkaline phosphatase, [wherein] comprising the step of expressing the enzyme [is expressed] in a host strain as claimed in one of the claims 11 to 14.

Claims

1. A process for the production of a eukaryotic alkaline phosphatase in yeast cells comprising the steps: a) cloning an alkaline phosphatase gene sequence into different vectors b) transformation of the yeast, c) expression of the alkaline phosphatase and d) purification of the alkaline phosphatase, wherein
   - a first vector is utilized that has a resistance gene for a first selection marker,
   - transformants are used which have integrated the resistance gene and the gene sequence into the genome, said transformants being selected by growth on nutrient medium containing a low concentration of a first selection marker,
   - the gene copy number is increased by multiple transformation in which multiple transformants are selected by growth on nutrient medium at an increased selection pressure,
   - a second vector is used which has a resistance gene for a second selection marker,
   - the gene copy number is increased by multiple transformation with the second vector in which multiple transformants are selected by growth on nutrient medium at an increased selection pressure and
   - those tranformants which have integrated several copies of the gene sequence and the selection marker resistance genes in a stable manner are selected for producing the eukaryotic alkaline phosphatase.

2. The process according to claim 1, wherein the gene sequence for the alkaline phosphatase corresponds to SEQ ID NO:1.

3. The process as claimed in claim 1, wherein the alkaline phosphatase gene sequence corresponds to SEQ ID NO:5.

4. The process as claimed in claim 1, wherein methylotrophic yeast cells are used.

5. The process as claimed in claim 1, wherein Pichia pastoris or Hansenula polymorpha is used as the yeast strain.

6. The DNA sequence according to SEQ ID NO:5.

7. A transformation vector containing SEQ ID NO:5.

8. The vector as claimed in claim 7, which essentially corresponds to pHAP10-3.

9. A vector comprising the expression cassette from pHAP10-3.

10. The vector as claimed in claim 9, which essentially corresponds to pHAP10-3/9K.

11. A host strain transformed with a vector as claimed in claim 9 or 10.

12. A host strain transformed with the vector pHAP10-3/9K and/or a vector as claimed in claims 7 or 8.

13. The host strain as claimed in claim 12, wherein Pichia pastoris or Hansenula polymorpha is used as the host strain.

14. A Pichia pastori X-33 yeast strain transformed with a vector as claimed in claims 8 to 10.

15. A process for producing a eukaryotic highly active alkaline phosphatase, comprising the step of expressing the enzyme in a host strain as claimed in one of the claims 11 to 14.